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Hepatic bile acid uptake in humans and mice: Multiple pathways and expanding potential role for gut-liver signaling

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Hepatic bile formation depends upon the synthesis, canalicular secretion, and return of bile acids (BA) in the enterohepatic circulation. Hepatocellular uptake of BAs from sinusoidal blood is mediated by a combination of transporters including the Na⁺-Taurocholate cotransporting polypeptide (NTCP; gene symbol *SLC10A1*) and members of the Organic Anion Transporting Polypeptide (OATP/*SLCO*) family of facilitative carriers such as OATP1B1 and OATP1B3 in humans and Oatp1b2 in mice. Although the identity of these carriers has been known for many years, recent studies of patients and mouse models are providing important new insights to their relative quantitative significance, as well as the role of gut-liver signaling in their regulation.

Based upon its expression and transport properties, the NTCP was long presumed the major mechanism for hepatic clearance of conjugated BAs. This tenet was borne out in 2015 when a pediatric patient was described with an inherited functional defect in NTCP and highly elevated serum levels of conjugated BAs.¹ Notably, this patient exhibited no jaundice, pruritus, or other signs of liver disease, raising important questions regarding the long-term pathophysiologic consequences of reduced hepatic BA clearance. Since then, two additional reports of patients with NTCP deficiency have appeared, which are beginning to answer those questions.^{2,3} The first report describes a Chinese pediatric patient with transient jaundice and a marked sustained hypercholanemia, where plasma BAs reached >700 μmol/L (normal: 0–10 μmol/L) over the first 30 months of life.² Similar to the initial case report, there was no obvious clinical phenotype and the child had normal anthropomorphic indices without signs of delayed motor milestones or growth retardation. The patient is homozygous for a c.800C>T nonsynonymous single nucleotide polymorphisms (rs2296651; p.Ser267Phe), which is prevalent in Asian populations (Minor allele frequency > 5%).^{4,5} In cells, this amino acid change leads to near complete loss of BA transport activity and also

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reduces HBV infection.⁴ Screening of an additional group of healthy individuals in Guangzhou, China identified more heterozygous carriers, as well as a homozygous asymptomatic 30-year-old female subject with only mildly elevated serum BAs (19.3 $\mu\text{mol/L}$). The second new report describes a 30-year old female patient of Thai origin with moderately elevated serum BA levels (values less than 90 $\mu\text{mol/L}$). The patient is a compound heterozygote, carrying the c.800C>T variation (p.Ser267Phe) and a frameshift mutation (c.615-618del; pSer206Profs*12) on the other allele, and showed normal weight, height, and cognitive development. The patient is apparently asymptomatic except for recurrent urticarial rash, but was identified as a result of late fetal loss. In light of the association of high BAs and adverse pregnancy outcomes in Intrahepatic Cholestasis of Pregnancy, it is possible that the patient's hypercholanemia may have contributed to the late fetal demise. Although the number of subjects is very small, the finding of only mild to moderate serum BA elevations in the NTCP-deficient adults suggests potential adaptations, which study of the NTCP-deficient mouse is beginning to address.

In 2015, Slijepcevic et al reported the phenotypic characterization of an NTCP (*Slc10a1*) knockout mouse.⁶ Surprisingly, more than half of the adult NTCP knockout mice maintained normal plasma levels of conjugated BAs, suggesting the presence of alternate hepatic bile acid clearance mechanisms. Notably, the hypercholanemic mice expressed lower hepatic levels of several Oatps, particularly Oatp1a1. In an encore performance, the authors used a combination of genetic and pharmacological approaches to elucidate those alternative clearance mechanisms.⁷ In humans, mutations in the adjacent OATP genes, *SLCO1B1* and *SLCO1B3*, are responsible for Rotor Syndrome, a benign conjugated hyperbilirubinemia. Similarly, knockout mice lacking the entire family of Oatp1a/Oatp1b (*Slco1a/1b*) transporters exhibit a marked conjugated hyperbilirubinemia and elevated plasma unconjugated but not conjugated BAs. In the present study, the authors examine the contribution of the murine Oatps to hepatic conjugated BA clearance in wild type and *Slco1a/1b* knockout mice treated with the NTCP inhibitor, myrcludex B. Whereas acute inhibition of NTCP with myrcludex B had only a small effect on plasma taurocholate clearance in wild type mice, it essentially abrogated taurocholate clearance and biliary secretion in *Slco1a/1b* knockout mice. Plasma conjugated BA levels were also marked elevated in those mice, affirming a critical compensatory role for the Oatps. The availability of *Slco1a/1b* knockout mice expressing the human OATP1B1 then permitted the investigators to begin examining whether human OATPs can play a similar compensatory role. This was particularly important in light of the relatively mild hypercholanemia recently reported for adult human subjects with NTCP deficiency. Although hepatic expression of human OATP1B1 reversed the conjugated hyperbilirubinemia in *Slco1a/1b* knockout mice (as reported previously), it was not able to compensate and block the rise in plasma conjugated BAs after inhibition of NTCP with myrcludex B. Moreover, administration of myrcludex B to healthy human subjects for 6 days significantly elevated plasma BA levels.

The findings provided an explanation for why plasma BA levels were normal in most of the NTCP knockout mice. But it did not explain why hepatic Oatp expression was reduced in the subset of NTCP knockout mice developing hypercholanemia. Although inflammation can reduce hepatic Oatp1a1 expression, cytokine levels were not increased nor were these signs of hepatic inflammation in the hypercholanemic mice. But interestingly, hepatic BA

synthesis, as estimated by measuring plasma 7 α -hydroxy-4-cholesten-3-one (C4) levels, was significantly reduced in NTCP knockout mice, suggesting increased gut-liver signaling via the ileal FXR-FGF15/19 - hepatic FGFR4 pathway. Indeed, ileal expression of FGF15 (human ortholog, FGF19) was increased and hepatic expression of Cyp7a1 reduced in the hypercholanemic myrcludex B-treated *Slco1a1/1b* knockout mice. With regard to ileal FGF15 expression, the same trend was observed in hypercholanemic NTCP knockout mice and in bile duct-ligated wild type mice treated with myrcludex B, leading the authors to hypothesize that BAs can be internalized by enterocytes from the basolateral (blood) side by the bidirectional transporter, OST α -OST β , when BA concentrations are sufficiently elevated. By this mechanism, the ileum can function to sense plasma BA levels, and respond with production of the enterokine FGF15/19 to suppress hepatic BA synthesis. Moreover, additional evidence was presented suggesting that this gut-liver signaling and FGF15 may be mediating the suppression of hepatic Oatp expression in the NTCP knockout mice.

Overall, the results indicate that both NTCP and Oatps participate in hepatic clearance of conjugated BAs in mice. Whether the hepatic OATPs can partially compensate for loss of NTCP in humans, particularly in adults is still unclear. Mean plasma BA levels were significantly increased in human subjects after administration of myrcludex B, but there was considerable variation in the response, with some subjects exhibiting only mild elevations. The findings also build upon the concept of the ileal enterocyte as a BA sensor. BA signaling in the gut-liver axis is widely recognized, whereby BAs internalized from the gut lumen by the ASBT act through FXR-FGF15/19 to regulate hepatic BA synthesis and other metabolic pathways. In this new twist, ileal enterocyte signaling pathway may also be engaged under conditions of very high plasma BA levels. Additional studies such as analysis of *Slco1a1/1b* knockout mice lacking OST α -OST β will be needed to directly explore those mechanisms. Finally, studies such as this work by Slijepcevic *et al* will be needed to understand the mechanisms that modify the presentation of NTCP deficiency in humans and ultimately the role of BAs in health and disease.

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Abbreviations

ASBT	apical sodium-dependent bile acid transporter
BA	bile acid
C4	7 α -hydroxy-4-cholesten-3-one
FGF15	fibroblast growth factor 15
FGFR4	fibroblast growth factor receptor-4
FXR	farnesoid X-receptor

NTCP	Na ⁺ -taurocholate cotransporting polypeptide
OATP	organic anion transporting polypeptide
OST	organic solute transporter

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Fig 1.

Working model of the phenotype associated with mouse NTCP deficiency. ASBT, apical sodium-dependent bile acid transporter; BSEP, bile salt export pump; FGF15, fibroblast growth factor-15; OATP, organic anion transporting polypeptide; OST, organic solute transporter; T-BA, taurine-conjugated bile acid.